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Alterations in brain monoamine neurotransmitter release at high pressure

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Summary. High pressure exposure produces neurological changes which manifest as tremors, EEG changes and convulsions. Since previous studies have implicated the involvement of the monoaminergic system in these symptoms, it was of interest to study monoamine release at high pressure. Synaptosomes isolated from guinea pig brain were used to follow monoamine efflux at 68 ATA. The major observation was a decrease in the initial calcium dependent release of all three monoamines in response to K^+ induced depolarization. This response is similar to that previously observed for GABA, glycine and glutamate. This generalized pressure induced depression of initial transmitter release suggests a mechanism common to the release process for both excitatory and inhibitory neurotransmission.

Key words: Neurotransmitter – High pressure nervous syndrome – Synaptosome – Monoamine – Pressure

Introduction

High hydrostatic pressure is the etiologic factor underlying nervous system changes referred to as the high pressure nervous syndrome (HPNS). HPNS is observed when human divers and experimental animals are exposed to pressures greater than 27 atmospheres absolute (ATA). The symptoms of HPNS resemble to varying degrees other neurological dysfunctions such as the metabolic encephalopathies, Parkinson's Syndrome, MPTP toxicity, or reaction to the street drug "Ecstacy" (Barnes 1988; Bennett and McLeod 1984; Hallenbeck 1981; Hiekkila 1988; Langston et al. 1983; Ricaurte et al. 1985; Vaernes et al. 1983). The disorder is progressive and appears in several stages. Initial manifestations are in the form of neuromuscular tremors succeeded by EEG changes, myoclonus and finally convulsions (Hunter and Bennett 1974).

A possible role of the brain monoamines (i.e., serotonin, dopamine, and norepinephrine) in the development of HPNS has been speculated for some time. Brauer et al. (1978) first showed that reserpine, a monoamine depleting alkaloid, accentuated pressure-induced convulsions in mice. The reserpine effect could be partially reversed by the administration of a monoamine precursor or a monoamine oxidase inhibitor. Subsequently, Koblin et al. (1980) found that FLA-63, a dopamine-β-hydroxylase inhibitor that depletes brain norepinephrine, caused a significant reduction in the pressure at which tremors and convulsions occurred. In a later study, Bowser-Riley et al. (1982) investigated the monoamine and high pressure interaction using neurotoxins that cause physical destruction of monoaminergic neurons. They found that those agents which depleted norepinephrine also consistently lowered the HPNS threshold level. In contrast, the depletion of brain serotonin was found to have no effect on the HPNS pressure threshold. These findings led Bowser-Riley (1984) to conclude that, of the monoamines, only norepinephrine is associated with pressure-induced encephalopathy. On the other hand, some investigators feel that the hyperexcitability associated with pressure exposure results from a general imbalance of the three monoamine neurotransmitter systems (Koblin et al. 1980). Therefore, the present study was designed to characterize the effects of pressure on the release of [^{3}H]serotonin, [^{3}H]dopamine, and [^{3}H]norepinephrine from presynaptic nerve terminals isolated from guinea pig brain.

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The presynaptic vesicle (synaptosome) preparation was selected because it retains the transport properties of the pre-synaptic nerve ending in situ, responding to depolarization with a release of neurotransmitters (Blaustein et al. 1977; Cotman et al. 1976; Debelleroche and Bradford 1973). Synaptosomes also offer an advantage for studying neurotransmitter release since multi-neuronal or neuro-glial interactions can be eliminated.

Material and methods

Animals

Adult male Hartley guinea pigs (300–400 g) (Charles Rivers Breeding Lab, Willington, MA) were housed at the Laboratory Animal Facility, Naval Medical Research Institute, under a 12 h dark cycle with food and water provide ad libitum.

Material

Radioactive [^{3}H]serotonin [(5-^{3}H)-5-hydroxytryptamine creatinine sulfate; 15–30 Ci/mmol], [^{3}H]dopamine (specific activity = 20–40 Ci/mmol), and 1-[^{3}H]norepinephrine (5–15 Ci/mmol) were purchased from New England Nuclear Corporation (Boston, MA). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

Method

Guinea pigs were decapitated and the brain rostral to the cerebellum placed in ice-cold 0.32 M sucrose buffered with HEPES. Synaptosomes were prepared by a previously described technique (Gilman et al. 1986a).

The method used for radioactive monoamine loading of the synaptosomes was similar to that previously described (Rutledge 1987; Steele et al. 1987). The final synaptosome pellet was suspended in 10 volumes of buffer medium. The composition was (in mM): NaCl, 145; KCl, 5; $CaCl_2$, 1.2; $MgCl_2$, 2.5; KH_2Po_4, 1.2; HEPES, 20; glucose, 10; pargyline, 0.01; pH 7.4. Aliquots (1 ml) of the synaptosomal preparation containing 3.0–3.5 mg/ml protein were incubated for 15 min at 37° C, and then the radio-labeled monoamine (1 µCi) added and incubation continued for 25 min to allow uptake by the tissue.

Compression studies

Following uptake, a 1 ml aliquot of the radioisotope-loaded synaptosome suspension was transferred to a filter unit consisting of a 0.45 µ nylon membrane filter positioned on a multiperforated support of a 10-ml perfusion chamber. The perfusion chamber was then connected to a peristaltic pump and the filters immediately washed with 25 ml of buffer medium using the highest pump speed.

After washing, the filter unit and superfusion apparatus were placed in a hyperbaric chamber (model 18361, Bethlehem, Hellertown, PA). Ten milliliters of buffer medium were poured directly over the filter and the hyperbaric chamber was flushed with 100% oxygen for 1 min, pressurized to 1.3 ATA with oxygen, and then to a final depth of 68 ATA with helium, at a compression rate of 4.03 ATA/min. Oxygen partial pressure as measured with a paramagnetic O_2 analyzer was maintained at 0.49 ± 0.1 ATA. A recirculation atmosphere control system was used to regulate CO_2 at < 0.0005 ATA, as measured by an infrared analyzer.

During compression, the filter was washed continuously with buffer medium. On reaching 68 ATA, the speed of the peristaltic pump was adjusted to perfuse the filters at a rate of 500 µl/min with buffer medium. Three "wash" perfusates were then collected directly into scintillation vials. At 3 min after the beginning of superfusion, the buffer medium was quickly substituted with a nondepolarizing (5 mM K^+), or a depolarizing (55 mM K^+) "efflux" buffer medium. The composition was (in mM): NaCl, 145 or 95; KCl, 5 or 55; $CaCl_2$, 1.2; $MgCl_2$, 2.5; KH_2PO_4, 1.2; HEPES, 20; glucose, 10; pH 7.4. Nine fractions containing 500 µl each of perfusate were collected every minute directly into scintillation vials.

During the release studies, the temperature of the buffer media and perfusion chamber was constantly monitored by microthermistors and constantly maintained at 37° C ± 0.05° C using YSI model 73A temperature control units. At the end of the release studies the hyperbaric chamber was decompressed to 1 ATA at the rate of 4.03 ATA/min. Aliquots from the same synaptosome preparation were used for obtaining the 1 ATA and 68 ATA release values for all experiments. The control preparations were handled in a manner similar to the experimental group, except that pressure exposures were in air at 1 ATA.

Fifteen ml of Biofluor was added to each scintillation vial and radioactivity determined for each perfusate. The radioactivity remaining on the filters at the end of the superfusion was also counted. Each filter was placed in a scintillation vial containing 500 µl of 1% sodium dodecylsulfate. After agitation, 15 ml of Biofluor was added and the filter counted for radioactivity.

Fractional efflux was expressed as percentage of total radioactivity, where total radioactivity was the sum of all fractional filtrate and radioactivity remaining on the filter. Data were analyzed by ANOVA and $P < 0.05$ was considered statistically significant.

Results

The time course of depolarization-induced efflux of [^{3}H]serotonin from synaptosome fractions isolated from the brain is shown in Fig. 1. Application of the high K^+ medium containing Ca^{2+} initially induced a four-fold increase in the release of [^{3}H]serotonin by the synaptosome preparation. After the first 2–3 min, however, this evoked release rapidly declined, returning within 8 min to the resting efflux levels.

Synaptosomes were also perfused with a depolarizing (55 mM K^+), calcium-free medium, with $MgCl_2$ replacing the omitted $CaCl_2$, to study the effect of calcium removal on depolarization-induced [^{3}H]serotonin release. The removal of calcium from the depolarizing medium essentially eliminated the four- to five-fold increase in serotonin release evoked by high K^+ (Fig. 1). Qualitatively similar responses were obtained for dopamine and norepinephrine (figures not shown).

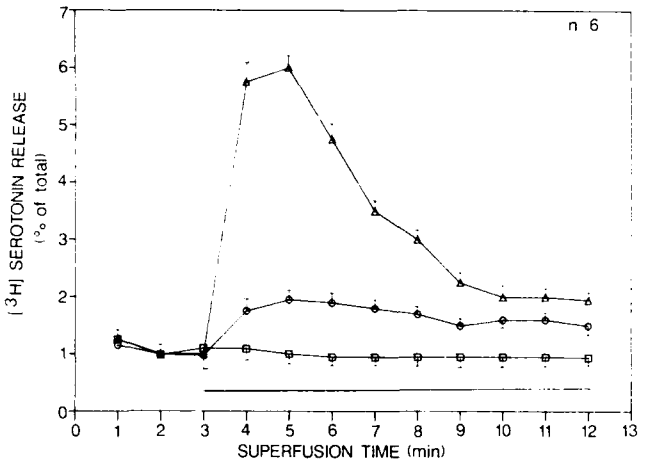

Fig. 1. Release of [^{3}H]serotonin from guinea pig brain synaptosome fractions. (△), depolarized, Ca^{2+} present; (○), depolarized, Ca^{2+} absent; (□), non-depolarized, Ca^{2+} present. After pre-loading with [^{3}H]serotonin, synaptosome aliquots were superfused with a high sodium, calcium-free buffer medium for 3 min, then 55 mM K^+-containing medium with or without Ca^{2+} or 5 mM K^+-containing medium with Ca^{2+} was similarly superfused for the 9 min marked by horizontal bar (i.e., 3–12 min). Each point represents mean ± SEM of six experiments for each condition

Pressurization of the synaptosome preparation to 68 ATA did not cause any significant change ($p > 0.05$) in the resting, non-depolarized efflux of serotonin, dopamine, or norepinephrine by these pre-synaptic nerve terminals. However, the total evoked release (i.e., both calcium-dependent and calcium-independent) of either [^{3}H]serotonin, [^{3}H]dopamine, or [^{3}H]norepinephrine from synaptosomes in the presence of 1.2 mM Ca^{2+} was suppressed by compression to 68 ATA (Figs. 2A, 3A and 4A). Data analysis showed that the depression in release of all 3 monoamines were significant ($p > 0.05$) at the first minute after potassium depolarization. The release of the three monoamines in the absence of Ca^{2+}, on the other hand, was little affected by compression ($p > 0.05$) (Figs. 2B, 3B, 4B for [^{3}H]serotonin. The difference in release between the Ca^{2+} containing and Ca^{2+} free media, which is the calcium-dependent component of release, is similar to the total release profile since the calcium-independent component was negligible (Figs. 2C, 3C, 4C). Statistical comparison between the 1 ATA control calcium-dependent data and the 68 ATA calcium-dependent data indicates that release of the [^{3}H]serotonin and [^{3}H]norepinephrine during the first two minutes after potassium depolarization is significantly lower at high pressure than in corresponding control values ($p < 0.05$). Dopamine release, on the other hand, was reduced

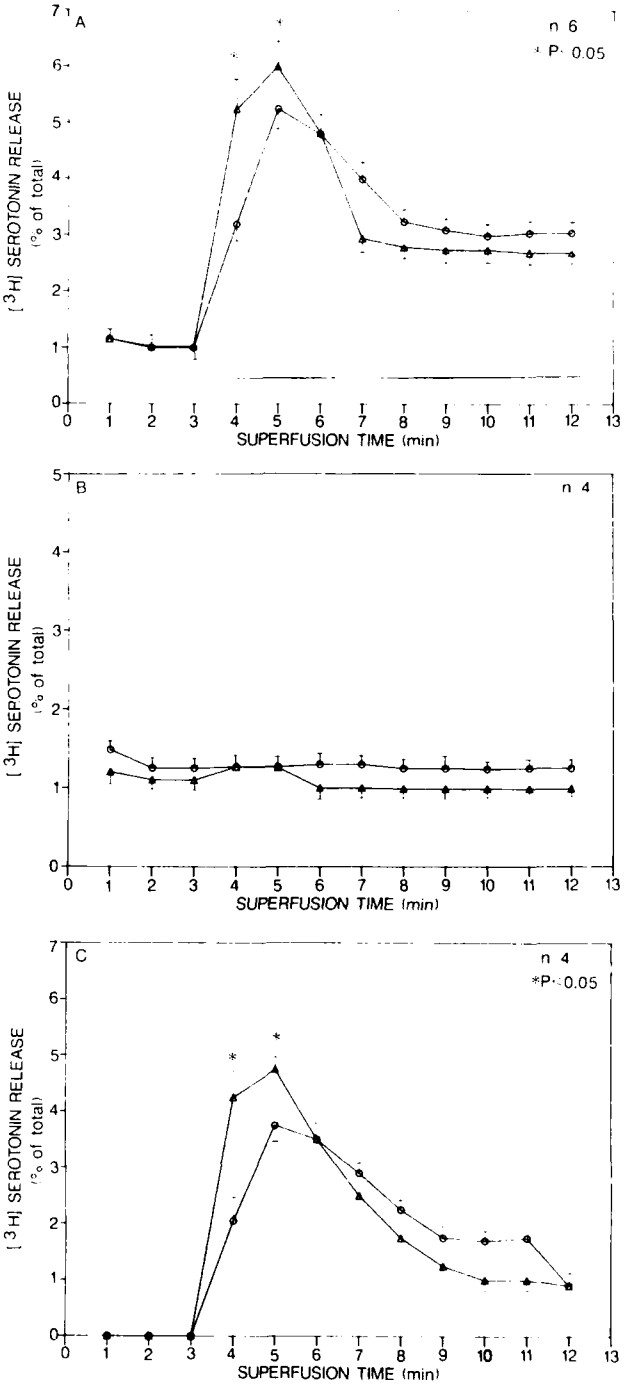

Fig. 2A–C. Effect of compression to 68 ATA on the high K^+-evoked release of [^{3}H]serotonin from synaptosomes. **A** In the presence of Ca^{2+}; **B** in the absence of Ca^{2+}; **C** Ca^{2+}-dependent release, i.e., the difference between **A** and **B**; (△), 1 ATA; (○), 68 ATA

only during the first minute of pressure exposure. This indicates that compression is selectively depressing the initial depolarization-induced calcium-dependent release of the [^{3}H]monoamines by synaptosomes isolated from the CNS.

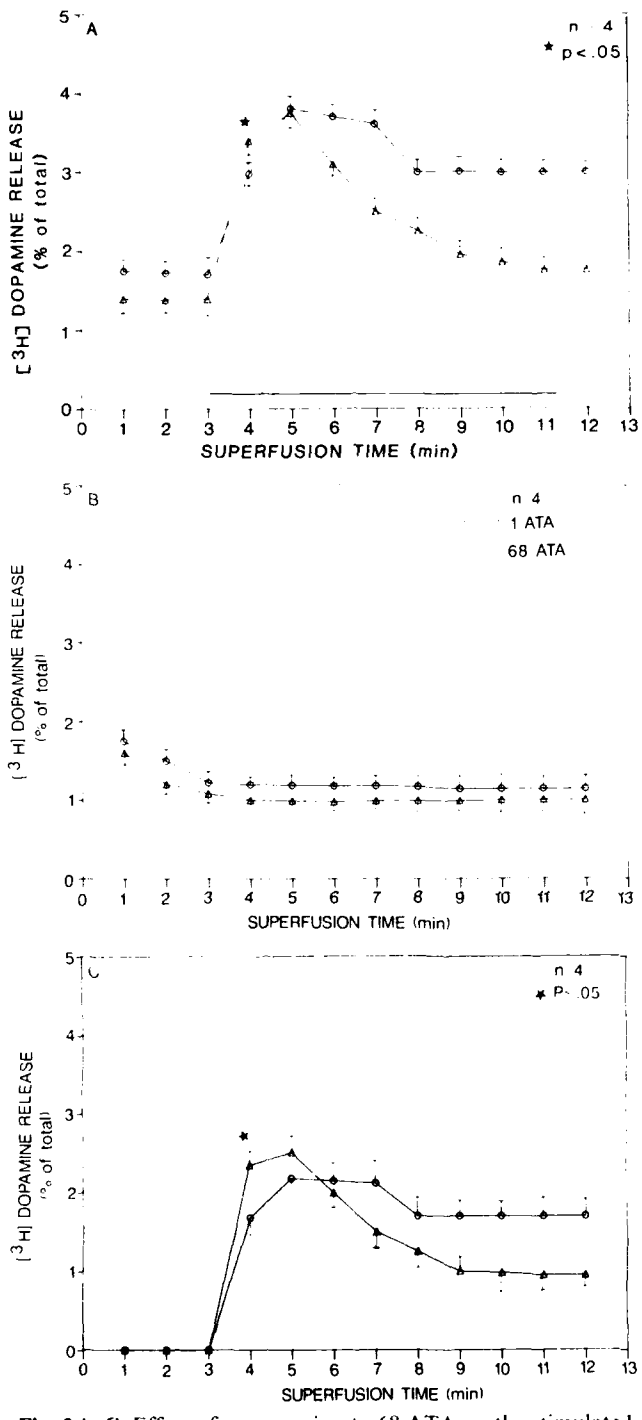

Fig. 3A–C. Effect of compression to 68 ATA on the stimulated release of [^{3}H]dopamine **A**, in the presence of Ca^{2+}; **B** in the absence of Ca^{2+}; **C** Ca^{2+}-dependent release; (△), 1 ATA; (○), 68 ATA. Each point represents mean ±SEM of four experiments

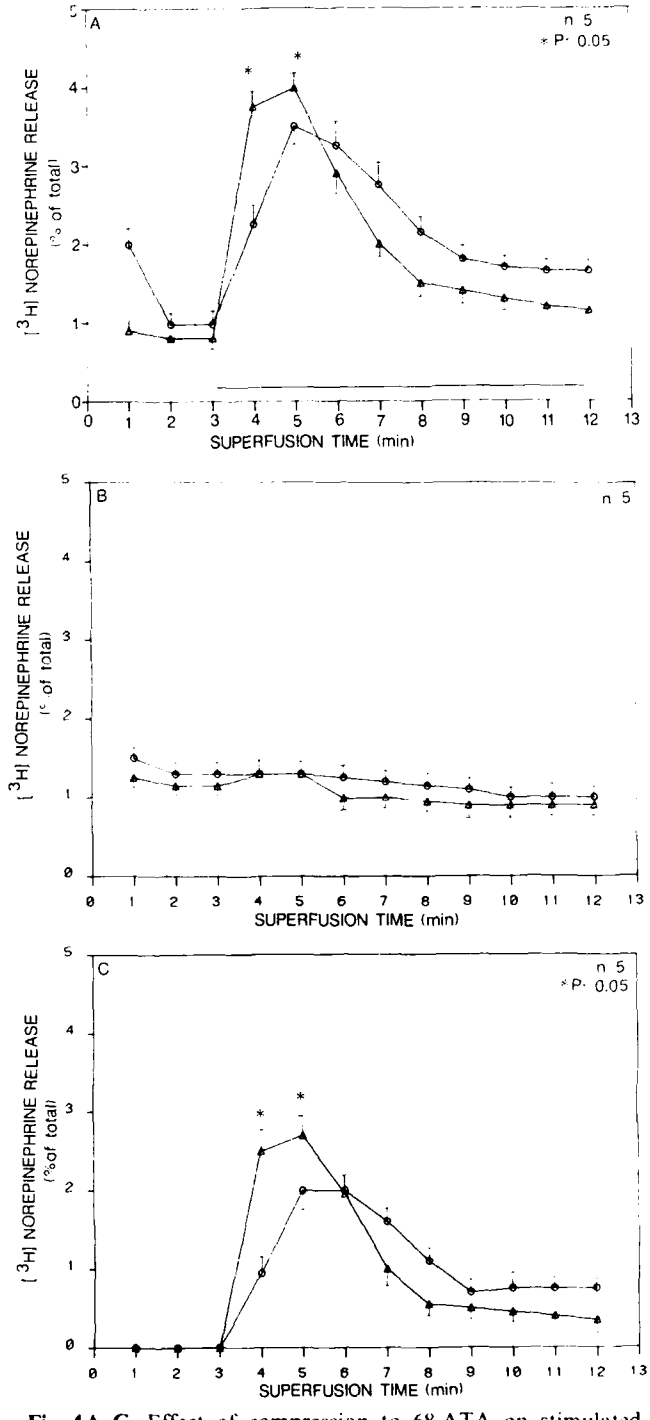

Fig. 4A–C. Effect of compression to 68 ATA on stimulated [^{3}H]norepinephrine release; **A** in the presence of Ca^{2+}; **B** in the absence of Ca^{2+}; **C** Ca^{2+}-dependent release; (△), 1 ATA; (○), 68 ATA

Discussion

High pressure nervous syndrome is associated with clinical manifestations that partially resemble the outward signs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxication, Parkinson's Syndrome, and 3,4-methylenedioxymeth-amphetamine (MDMA) administration (i.e., muscle tremor, sleep difficulty, impaired cognition, and reduced psychomotor performance) (Bennett and

McLeod 1984; Hallenbeck 1981; Vaernes et al. 1983). As previously mentioned, reserpine pretreatment in mice produces a significant decrease in brain serotonin, dopamine, and norepinephrine, and is associated with a fall in tremor threshold when these animals are exposed to high pressure (Koblin et al. 1980). These observations suggest that depression of monoaminergic pathways may contribute to the outward clinical signs of high pressure exposure.

The major observation in this study was a significant suppression in the initial (i.e., the first 1 or 2 min) efflux of the three monoamines (viz. serotonin, dopamine, and norepinephrine) from depolarized brain synaptosomes after compression to 68 ATA. This suppression of stimulated release coincides with decreases previously noted in the calcium-dependent release for GABA, glycine, and glutamate after pressurization to 68 ATA (Gilman et al. 1986b; Gilman et al. 1987). Thus, the effect of high pressure on potassium-stimulated, calcium-dependent transmitter efflux in perfused synaptosomes, appears to produce a generalized retardation in the initial release of all the transmitter substances mentioned above.

Since there appears to be a generalized pressure-induced inhibition of initial transmitter release from potassium depolarized synaptosomes, it is reasonable to suspect a common factor affecting the mechanism of release for most (if not all) calcium-dependent neurotransmitters. It is not clear whether release is being depressed as a consequence of an impairment of calcium entry into the nerve terminal or as the result of an interruption of some other aspect of the calcium-dependent cascade that couples stimulation with release (e.g., calcium-calmodulin binding, membrane phosphorylation, or vesicle fusion) (Augustine et al. 1987; DeLorenzo 1980; Huttner et al. 1983; Heinemann et al. 1987; Pollard et al. 1987).

In an earlier study, we have observed that $^{45}Ca^{2+}$ uptake into synaptosomes is significantly depressed at 68 ATA (Gilman et al. 1986a). This finding strengthens the supposition that high pressure most likely alters voltage-sensitive calcium channel gating, and therefore depresses transmitter release. On the other hand, there is some evidence that other components of the cascade for calcium-dependent transmitter release are also modified by elevated pressure. Support for this notion is provided by studies in our laboratory with the calcium ionophore, A23187, applied to synaptosomes prior to exposing them to elevated pressure. Preliminary data reveals that A23187 does not reverse the pressure-induced inhibition of calcium-dependent transmitter release. Since A23187 forms a membrane-bound calcium – hydrogen ion exchange carrier, the endogenous membrane calcium channel should be bypassed. Thus, calcium entry into the terminal should occur independently of the endogenous calcium channel. The A23187 results, therefore, lead us to suspect a pressure-induced modification of components in the release subsequent to calcium entry. However, it is currently unknown which component(s) is(are) involved. Further support for this supposition is provided by the studies of Heinemann et al. 1987. These investigators observed that Ca^{2+} currents in patch-clamped chromaffin cells were independent of pressures up to 400 atm and that the release process was slowed down while a decrease in membrane capacitance was noted. This suggests that pressure affects neurotransmitter release at some point other than the calcium ion channel.

The depression of the early phase of transmitter release from nerve terminals in response to high pressure in the whole brain would have profound effects on information processing, timing, and modulatory action in motor, sensory, and behavioral pathways. However, it is unknown whether there is actually an analogous pressure-related depression of depolarization-induced, calcium-dependent release of serotonin, dopamine, or norepinephrine in the whole, intact brain. It is also unknown if this phenomenon is actually a contributory factor in the development of the outward symptomatology of HPNS. On the other hand, such a depression could contribute to the motor and behavioral changes associated with high pressure exposure.

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